

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (withdrawn) A megakaryocyte differentiation factor having the following properties:

1) stimulating differentiation of megakaryocytes;
2) exhibiting a molecular weight of 55 to 57 kD as determined by gel filtration and SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and having no intermolecular disulfide linkage;
3) exhibiting an isoelectric point of 6.5 ± 0.5 ; and
4) having at least one of the amino acid sequences shown in SEQ ID NO: 1 to 9 in the Sequence Listing.

2. (withdrawn) A megakaryocyte differentiation factor according to claim 1, produced by human cells.

3. (withdrawn) A megakaryocyte differentiation factor according to claim 2, produced by human cancer cells.

4. (withdrawn) A megakaryocyte differentiation factor according to claim 3, produced by cells derived from human epidermoid carcinoma cell A431.

5. (withdrawn) A megakaryocyte differentiation factor according to claim 4, produced by cells derived from human epidermoid carcinoma cell A431, cultured in a protein-free medium.

6. (withdrawn) A megakaryocyte differentiation factor having an amino acid sequence the same as the native amino acid sequence of a megakaryocyte differentiation factor according to claim 1, an amino acid sequence wherein one or more than one amino acid residue in said native amino acid sequence is deleted, an amino acid sequence wherein one or more than one amino acid residue in said native amino acid sequence is replaced with other amino acids, an amino acid sequence wherein one or more than one amino acid is added to said native amino acid sequence, or an amino acid sequence including a combination of said amino acid modifications.

7. (withdrawn) A megakaryocyte differentiation factor essentially consisting of the amino acid sequence shown in SEQ ID NO: 30.

8. (withdrawn) A megakaryocyte differentiation factor having an amino acid sequence wherein one or more than one amino acid residue in the amino acid sequence shown in SEQ ID NO: 30 is deleted, an amino acid sequence wherein one or more than one amino acid residue in the amino acid sequence shown in SEQ ID NO: 30 is replaced with other amino acids, or an amino acid sequence wherein one or more than one amino acid is added to the amino acid sequence shown in SEQ ID

NO: 30 or an amino acid sequence including a combination of said amino acid modification.

9. (withdrawn) A megakaryocyte differentiation factor according to claim 6, which is glycosylated.

10. (withdrawn) A megakaryocyte differentiation factor according to claim 7, which is glycosylated.

11. (withdrawn) A megakaryocyte differentiation factor according to claim 8, which is glycosylated.

12. (withdrawn) A megakaryocyte differentiation factor obtainable from a culture of transformed cells constructed by a gene recombination technique using a polynucleotide hybridizable with a polynucleotide coding for at least one of the amino acid sequences shown in SEQ ID NO: 1 to 9.

13. (withdrawn) A megakaryocyte differentiation factor according to claim 6, wherein the N-terminus of the factor is biochemically or chemically modified, preferably the first methionine is deleted and the second alanine is acetylated.

14. (withdrawn) A megakaryocyte differentiation factor according to claim 8, wherein the N-terminus of the factor is biochemically or chemically modified, preferably the first methionine is deleted and the second alanine is acetylated.

15. (withdrawn) A megakaryocyte differentiation factor according to claim 12, wherein the N-terminus of the factor is biochemically or chemically modified, preferably the first methionine is deleted and the second alanine is acetylated.

16-21. (canceled)

22. (withdrawn) A pharmaceutical composition comprising a megakaryocyte differentiation factor according to claim 1 and a pharmaceutically acceptable carrier.

23. (withdrawn) A pharmaceutical composition comprising a megakaryocyte differentiation factor according to claim 6 and a pharmaceutically acceptable carrier.

24. (withdrawn) A pharmaceutical composition according to claim 22, for treating thrombocytopenia.

25. (withdrawn) A pharmaceutical composition according to claim 23, for treating thrombocytopenia.

26. (withdrawn) An antibody to megakaryocyte differentiation factor according to claim 1.

27. (withdrawn) An antibody to megakaryocyte differentiation factor according to claim 6.

28-29. (canceled)

30. (previously presented) An isolated DNA coding for a megakaryocyte differentiation factor which stimulates differentiation of megakaryocytes, wherein said DNA hybridizes with a polynucleotide encoding the amino acid sequence of SEQ ID NO: 34.

31. (previously presented) A process for production of a megakaryocyte differentiation factor which stimulates differentiation of megakaryocytes, comprising the steps of culturing a eukaryotic or prokaryotic host cell transformed with an expression vector comprising a DNA encoding said megakaryocyte differentiation factor, wherein said DNA hybridizes with a polynucleotide coding for the amino acid sequence shown in SEQ ID NO: 34, under conditions suitable for producing the megakaryocyte differentiation factor, and recovering the megakaryocyte differentiation factor.

32. (previously presented) An isolated DNA coding for a megakaryocyte differentiation factor which stimulates differentiation of megakaryocytes, wherein said DNA hybridizes with a polynucleotide encoding the amino acid sequence of SEQ ID NO: 34, and wherein an amino acid sequence encoded by said DNA contains at least one of the amino acid sequences shown in SEQ ID NO: 1 to 9.

33. (previously presented) A process for production of a megakaryocyte differentiation factor which stimulates differentiation of megakaryocytes, comprising the steps of culturing a eukaryotic or prokaryotic host cell transformed with an expression vector comprising a DNA encoding said megakaryocyte differentiation factor, wherein said DNA hybridizes with a polynucleotide coding for the amino acid sequence shown in SEQ ID NO: 34, and wherein an amino acid sequence encoded by said DNA contains at least one of the amino acid sequences shown in SEQ ID NO: 1 to 9, under conditions suitable for producing the megakaryocyte differentiation factor, and recovering the megakaryocyte differentiation factor.

34. (new) The isolated DNA of claim 30, wherein the DNA hybridizes with the polynucleotide under conditions of 5X SSC, 25 mM phosphate buffer (pH 7.4), 5X Denhardt's solution, 1% SDS, 100 ug/ml heat denatured salmon sperm DNA, and 50% formate and 42°C for 18 hours, followed by washing in 5X SSC, 0.1% SDS at 40°C for 20 minutes.

35. (new) The process of claim 31, wherein the DNA hybridizes with the polynucleotide under conditions of 5X SSC, 25 mM phosphate buffer (pH 7.4), 5X Denhardt's solution, 1% SDS, 100 ug/ml heat denatured salmon sperm DNA, and 50% formate and 42°C for 18 hours, followed by washing in 5X SSC, 0.1% SDS at 40°C for 20 minutes.

36. (new) The isolated DNA of claim 32, wherein the DNA hybridizes with the polynucleotide under conditions of 5X SSC, 25 mM phosphate buffer (pH 7.4), 5X Denhardt's solution, 1% SDS, 100 ug/ml heat denatured salmon sperm DNA, and 50% formate and 42°C for 18 hours, followed by washing in 5X SSC, 0.1% SDS at 40°C for 20 minutes.

37. (new) The process of claim 33, wherein the DNA hybridizes with the polynucleotide under conditions of 5X SSC, 25 mM phosphate buffer (pH 7.4), 5X Denhardt's solution, 1% SDS, 100 ug/ml heat denatured salmon sperm DNA, and 50% formate and 42°C for 18 hours, followed by washing in 5X SSC, 0.1% SDS at 40°C for 20 minutes.